

2. Please delete the entire paragraph beginning on line 26 and continuing through line 29 on page 5, and replace with the following:

A2 "Figure 1 shows the viability of rabbit renal cortical slices subjected to simulated freezing in either DMSO (D) or Veg (V) at various concentrations. Viability is measured by tissue K/Na ratios after restoration of active metabolism. Each bar represents 6-12 individual slices."

IN THE CLAIMS:

Please CANCEL Claims 1-18.

Please ADD the following NEW Claims 19-45:

19. (NEW) A method for reducing or eliminating cooling injury in a living system, comprising:

435/325
A3 preparing a preservation medium having a tonicity which is sufficiently hypertonic to minimize cooling injury, wherein said preservation medium comprises a carrier solution and at least one cryoprotective agent which is sufficient in concentration to prevent freezing at a predetermined temperature below approximately 0°C;

adding said preservation medium to the living system; and

cooling the living system to said predetermined temperature.

20. (NEW) The method of Claim 19, wherein the tonicity is from 1 to 4 times isotonic.

21. (NEW) The method of Claim 19, wherein the tonicity is from 1.1 to 2.7 times isotonic.

22. (NEW) The method of Claim 19, wherein the tonicity is from 1.1 to 2 times isotonic.

23. (NEW) The method of Claim 19, wherein the tonicity is from 1.1 to 1.5 times isotonic.

24. (NEW) The method of Claim 19, wherein the tonicity is from 1.2 to 1.5 times isotonic.

25. (NEW) The method of Claim 19, wherein the tonicity of the preservation medium is increased by increasing the concentration of said carrier solution.

26. (NEW) The method of Claim 25, wherein the tonicity of the carrier solution is raised simultaneously with an increase in concentration of said cryoprotective agent.

27. (NEW) The method of Claim 26, wherein the tonicity of the carrier solution is increased by a proportion which is approximately equal to the proportional increase in the concentration of said cryoprotective agent.

28. (NEW) The method of Claim 27, further comprising reducing the tonicity of the preservation medium to isotonic while the concentration of said cryoprotective agent is diluted in similar proportion to the dilution of the carrier solution.

29. (NEW) A method for the rapid addition of at least one cryoprotective agent to a cell or tissue or organ, comprising:

adding a first amount of said cryoprotective agent to a carrier solution to produce a preservation medium under isotonic conditions; and

in one step, adding a second amount of said cryoprotective agent to produce a final amount of said cryoprotective agent and simultaneously increasing the concentration of the carrier solution such that the tonicity of the preservation medium is increased from isotonic to hypertonic, wherein the tonicity of the preservation medium increases by a proportion which is approximately equal to the proportional increase in the concentration of said cryoprotective agent attributable to the addition of the second amount of said cryoprotective agent to the first amount of said cryoprotective agent.

30. (NEW) The method of Claim 29, wherein the said final amount of said cryoprotective agent is rapidly removed by the simultaneous dilution of said cryoprotective agent and said carrier solution, and wherein the dilution is performed such that the relative concentration of the said final amount of cryoprotective agent and said carrier solution remains approximately the same.

31. (NEW) A method for reducing or eliminating cooling injury in a living system, comprising:

preparing a preservation medium having a tonicity which is sufficiently hypertonic to minimize cooling injury, wherein said preservation medium comprises a carrier solution, at least one cryoprotective agent, and at least one antinucleating polymer, wherein said cryoprotective agent and said antinucleating polymer are sufficient in concentration to prevent freezing at a predetermined temperature below approximately 0°C;

adding said preservation medium to the living system; and

cooling the living system to said predetermined temperature.

32. (NEW) The method of Claim 31, wherein the at least one antinucleating polymer is selected from the group consisting of polyglycerol, polyvinyl alcohol-polyvinyl acetate copolymer and a mixture thereof.

33. (NEW) A method for reducing or eliminating cooling injury in a living system, comprising:

preparing a preservation medium having a tonicity which is sufficiently hypertonic to minimize cooling injury, wherein said preservation medium comprises a carrier solution, at least one cryoprotective agent, and polyvinyl pyrrolidone, said polyvinyl pyrrolidone having a mean molecular mass of approximately 5000 daltons, wherein said cryoprotective agent and said polyvinyl pyrrolidone are sufficient in concentration to prevent freezing at a predetermined temperature below approximately 0°C;

adding said preservation medium to the living system; and

cooling the living system to said predetermined temperature.

I | 34. (NEW) A method for reducing or eliminating cooling injury in a living system, comprising:

preparing a preservation medium having a tonicity which is sufficiently hypertonic to minimize cooling injury, wherein said preservation medium comprises a carrier solution, at least one cryoprotective agent, and polyethylene glycol, said polyethylene glycol having a mean molecular mass of approximately 1000 daltons, wherein said cryoprotective agent and polyethylene glycol are sufficient in concentration to prevent freezing at a predetermined temperature below approximately 0°C;

Q3 adding said preservation medium to the living system; and

cooling the living system to said predetermined temperature.

35. (NEW) The method of Claim 19, wherein said at least one cryoprotective agent comprises dimethyl sulfoxide, formamide, and ethylene glycol.

36. (NEW) The method of Claim 19, wherein said at least one cryoprotective agent comprises dimethyl sulfoxide, formamide, and ethylene glycol, polyglycerol, and polyvinyl alcohol-polyvinyl acetate copolymer, and wherein the combination of polyglycerol and polyvinyl alcohol-polyvinyl acetate copolymer is at a total concentration of 0.1 to 0.7 times isotonic.

37. (NEW) The method of Claim 35, wherein the said cryoprotective agent further comprises acetol.

III | 38. (NEW) A method for reducing or preventing cooling injury in a living system, comprising:

preparing a first protective solution by adjusting the hypertonicity of the solution to be within a first tonicity range for minimizing cooling injury within a first predetermined temperature range;

preparing a second protective solution by adjusting the hypertonicity of the solution to be within a second tonicity range for minimizing cooling injury within a second predetermined temperature range;

contacting the living system with said first protective solution;

cooling the living system to a temperature within said first temperature range;

contacting the living system with said second protective solution;

cooling the living system to a temperature within said second temperature range.

Q3 39. (NEW) The method of Claim 38, wherein said first protective solution has a hypertonicity lower than the hypertonicity of said second protective solution.

40. (NEW) The method of Claim 38, wherein said first protective solution contains at least one antinucleating polymer.

41. (NEW) The method of Claim 38, wherein said second protective solution contains at least one antinucleating polymer.

42. (NEW) The method of Claim 38, wherein said first protective solution contains polyvinyl pyrrolidone having a mean molecular mass of approximately 5000 daltons.

43. (NEW) The method of Claim 38, wherein said second protective solution contains polyvinyl pyrrolidone having a mean molecular mass of approximately 5000 daltons.

44. (NEW) The method of Claim 38, wherein said first protective solution contains polyethylene glycol having a mean molecular mass of approximately 1000 daltons.

45. (NEW) The method of Claim 38, wherein said second protective solution contains polyethylene glycol having a mean molecular mass of approximately 1000 daltons.